

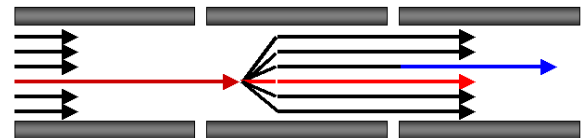


Skyline

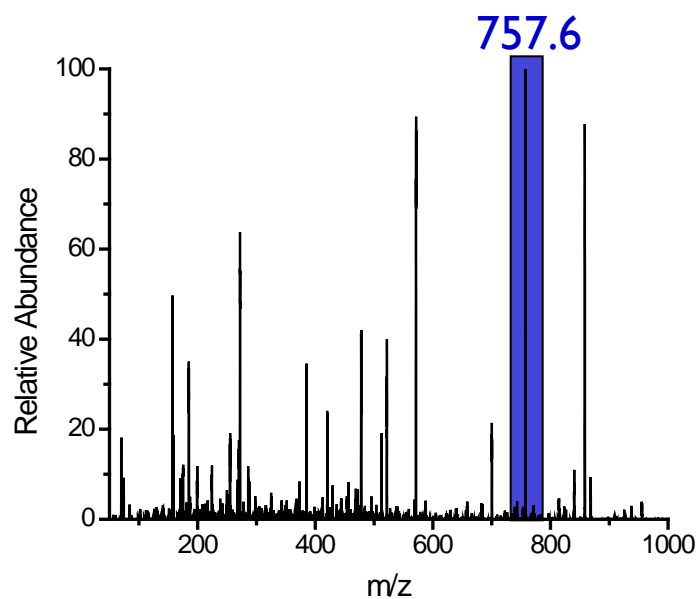
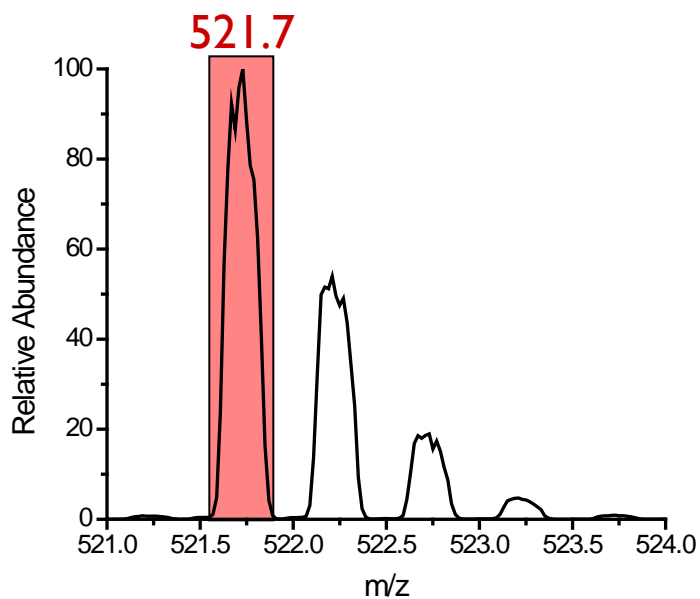
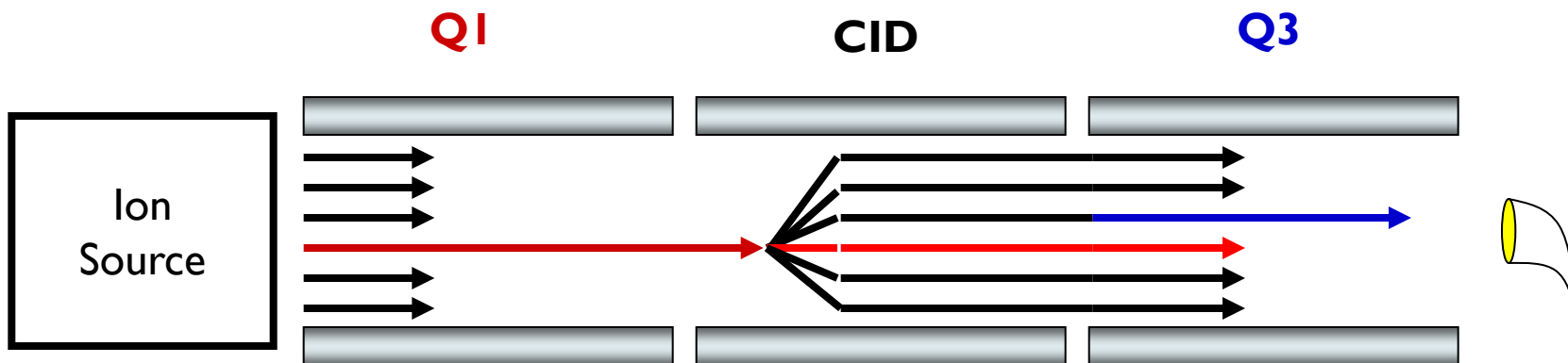
Targeted Proteomics Environment

Sharing SRM/MRM Method Creation and Results Analysis
Across Laboratories and Instrument Platforms

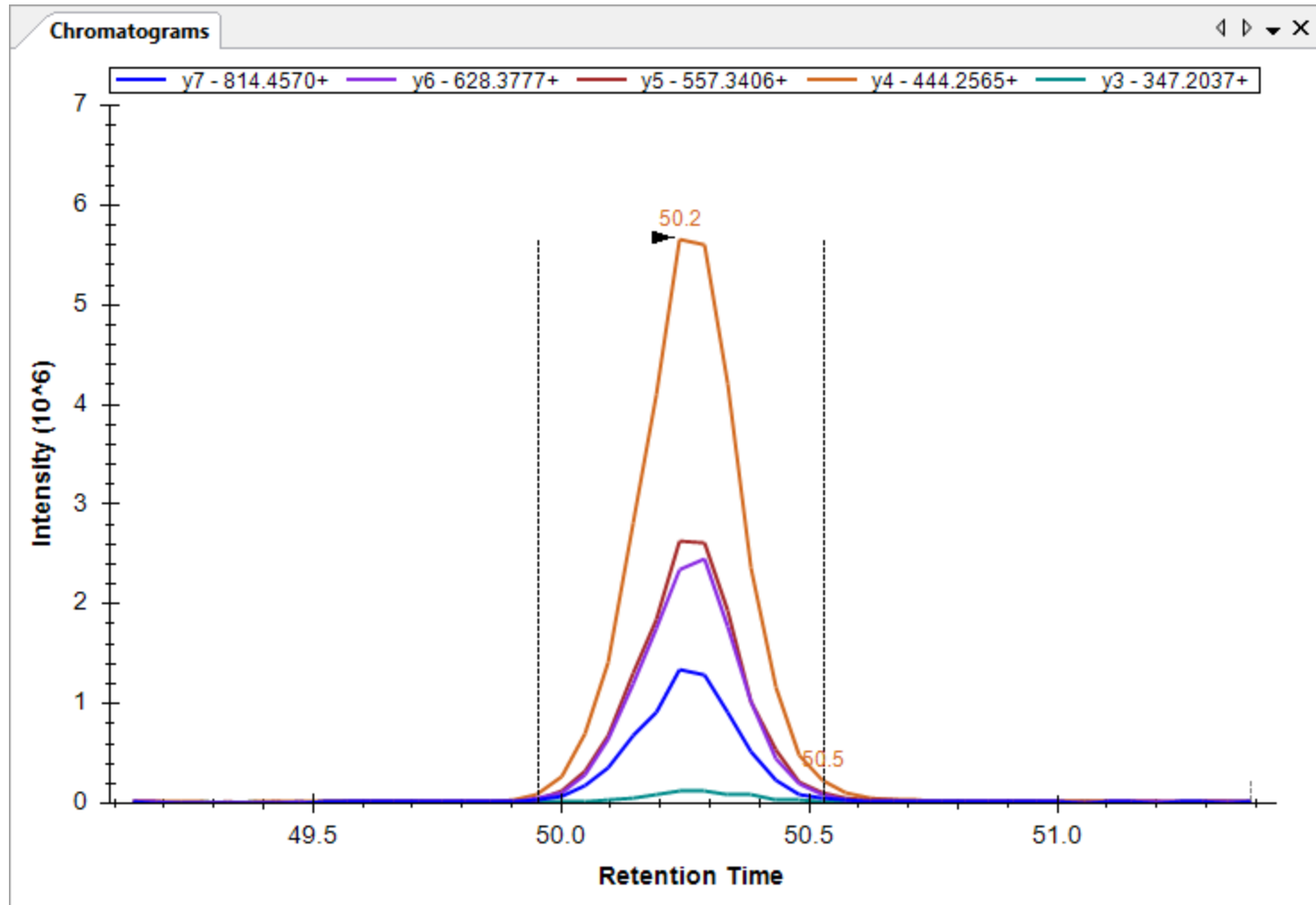
Brendan MacLean; Daniela Tomazela; Susan E. Abbatiello;
Birgit Schilling; Nicholas Shulman; Matthew Chambers;
David Tabb; Bradford Gibson; Steven A. Carr; Daniel C. Liebler;
Michael J. MacCoss



Selected Reaction Monitoring



Selected Reaction Monitoring



Spreadsheets

▶ NCI-CPTAC Study 7 summary of instrument set-up

	A	B	C	D	E	F	G	H	I	J	K	L
1	Protein	Signature Peptide*	Identifier	MH+	z	MRM Transitions		DP	CE	CXP	Fragment	
2				(mono)	(Q1)	Q1	Q3				Ion Type	
3	APR	AGLCQTFVYGGCR	bi0173	1488.7	2	744.8	858.39	120	35	16	y7	
4					2	744.8	959.44	120	38	16	y8	
5					2	744.8	1087.5	120	40	16	y9	
6		AGLCQTFVYGGCR	bi0081	1493.7	2	747.3	863.41	120	35	16	y7	
7					2	747.3	964.46	120	38	16	y8	
8					2	747.3	1092.5	120	40	16	y9	
9	LEP	INDISHTQSVSAK	bi0167	1399.7	3	467.2	586.8	61	20	12	y11 ²⁺	
10					3	467.2	643.82	61	20	12	y12 ²⁺	
11					3	467.2	720.39	61	20	12	y7	
12		INDISHTQSVSAK	ni0101	1407.3	3	469.9	590.81	61	20	12	y11 ²⁺	
13					3	469.9	647.83	61	20	12	y12 ²⁺	
14					3	469.9	728.4	61	20	12	y7	
15	MYO	LFTGHPETLEK	bi0171	1271.7	3	424.6	506.26	66	18	12	y9 ²⁺	
16					3	424.6	579.79	66	19	10	y10 ²⁺	
17					3	424.6	716.38	66	24	13	y6	
18		LFTGHPETLEK	ni0102	1279.7	3	427.2	510.27	66	18	12	y9 ²⁺	
19					3	427.2	583.8	66	19	10	y10 ²⁺	
20					3	427.2	724.4	66	24	13	y6	

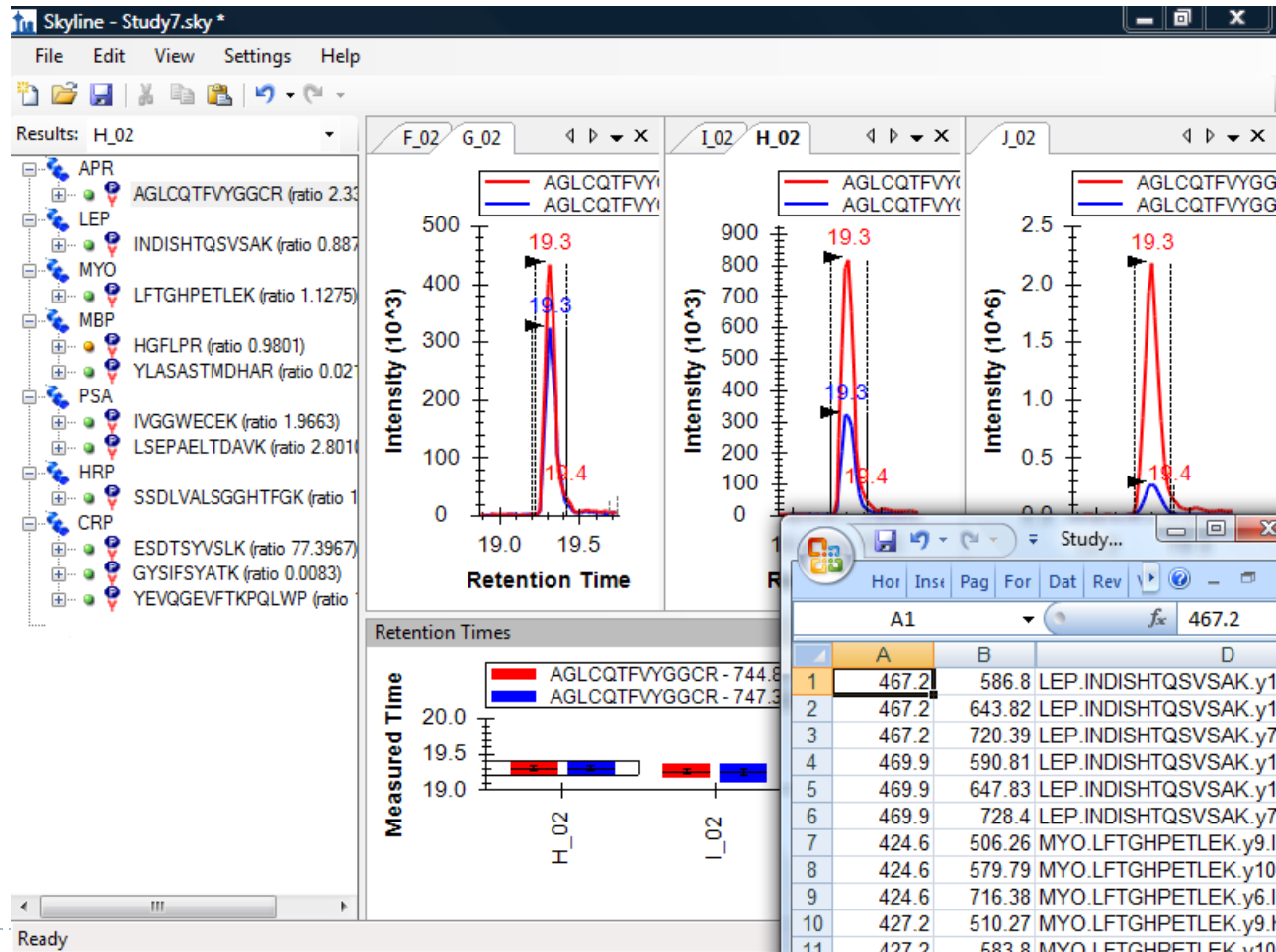
More Spreadsheets (66 transitions)

▶ NCI-CPTAC Study 7 raw transition list for 4000 Q Trap

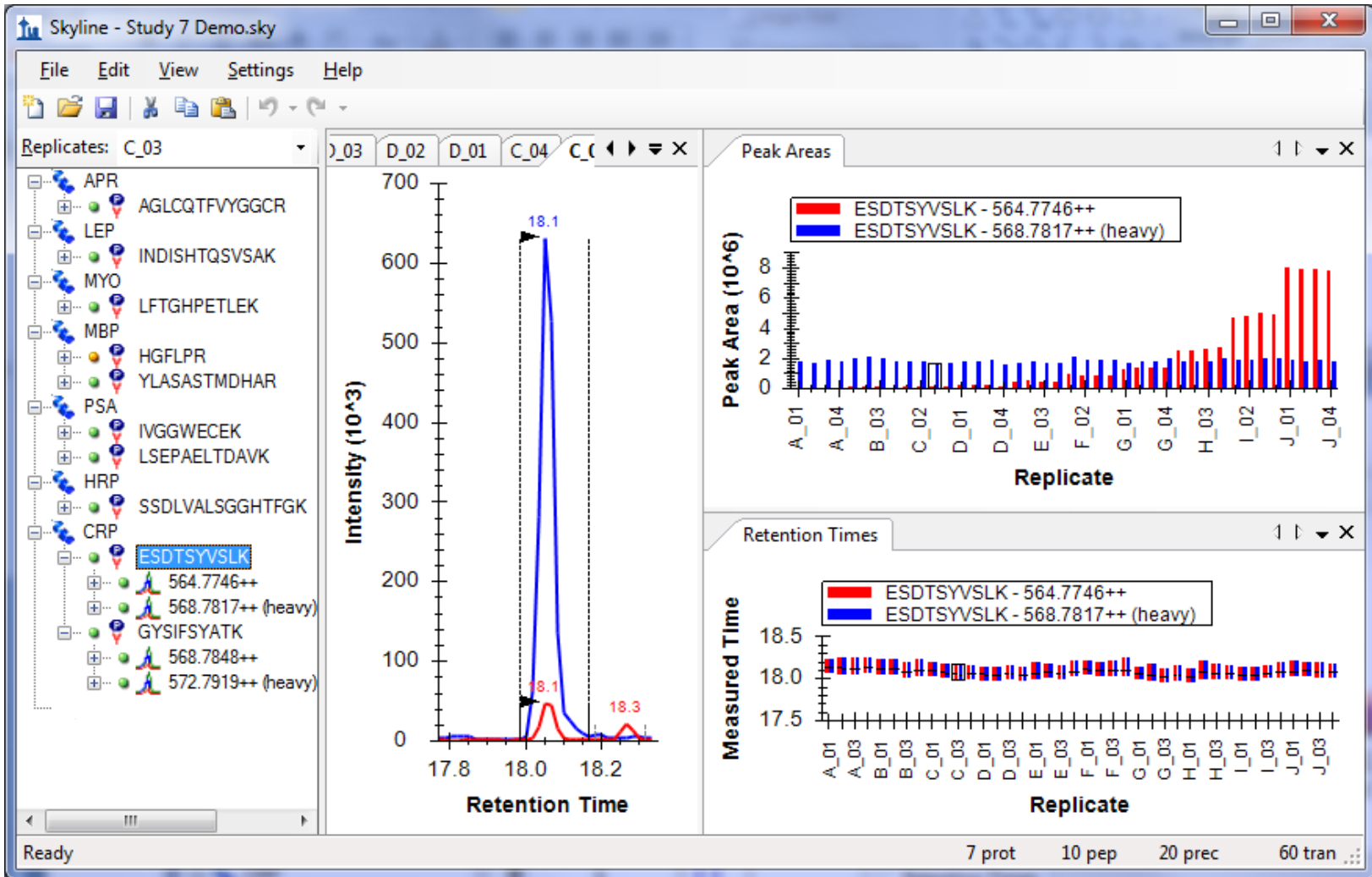
	A	B	C	D	E	F	G	H	I	J
1	744.8	858.39	10	APR.AGLCQTFVYGGCR.y7.light	105	40				
2	744.8	959.44	11	APR.AGLCQTFVYGGCR.y8.light	105	39				
3	744.8	1087.5	12	APR.AGLCQTFVYGGCR.y9.light	105	39				
4	747.3	863.41	13	APR.AGLCQTFVYGGCR.y7.heavy	105	40				
5	747.3	964.46	14	APR.AGLCQTFVYGGCR.y8.heavy	105	39				
6	747.3	1092.5	15	APR.AGLCQTFVYGGCR.y9.heavy	105	39				
7	467.2	586.8	16	LEP.INDISHTQSVSAK.y11.light	65	22				
8	467.2	643.82	17	LEP.INDISHTQSVSAK.y12.light	65	22				
9	467.2	720.39	18	LEP.INDISHTQSVSAK.y7.light	65	28				
10	469.9	590.81	19	LEP.INDISHTQSVSAK.y11.heavy	65	22				
11	469.9	647.83	20	LEP.INDISHTQSVSAK.y12.heavy	65	22				
12	469.9	728.4	21	LEP.INDISHTQSVSAK.y7.heavy	65	28				
13	424.6	506.26	22	MYO.LFTGHPETLEK.y9.light	65	19				
14	424.6	579.79	23	MYO.LFTGHPETLEK.y10.light	65	19				
15	424.6	716.38	24	MYO.LFTGHPETLEK.y6.light	65	23				
16	427.2	510.27	25	MYO.LFTGHPETLEK.y9.heavy	65	19				
17	427.2	583.8	26	MYO.LFTGHPETLEK.y10.heavy	65	19				
18	427.2	724.4	27	MYO.LFTGHPETLEK.y6.heavy	65	23				
19	363.7	385.26	28	MBP.HGFLPR.y3.light	70	23				
20	363.7	532.32	29	MBP.HGFLPR.y4.light	70	22				
21	363.7	589.35	30	MBP.HGFLPR.y5.light	70	23				
22	366.7	391.28	31	MBP.HGFLPR.y3.heavy	70	23				
23	366.7	538.34	32	MBP.HGFLPR.y4.heavy	70	22				
24	366.7	595.37	33	MBP.HGFLPR.y5.heavy	70	23				
25	441.5	488.22	24	MBP.VLASASTMDHAR.y0.light	60	24				

Protein and Peptide Centric Documents

- ▶ Import existing Study 7 experiment into Skyline



Graphic Display of Information

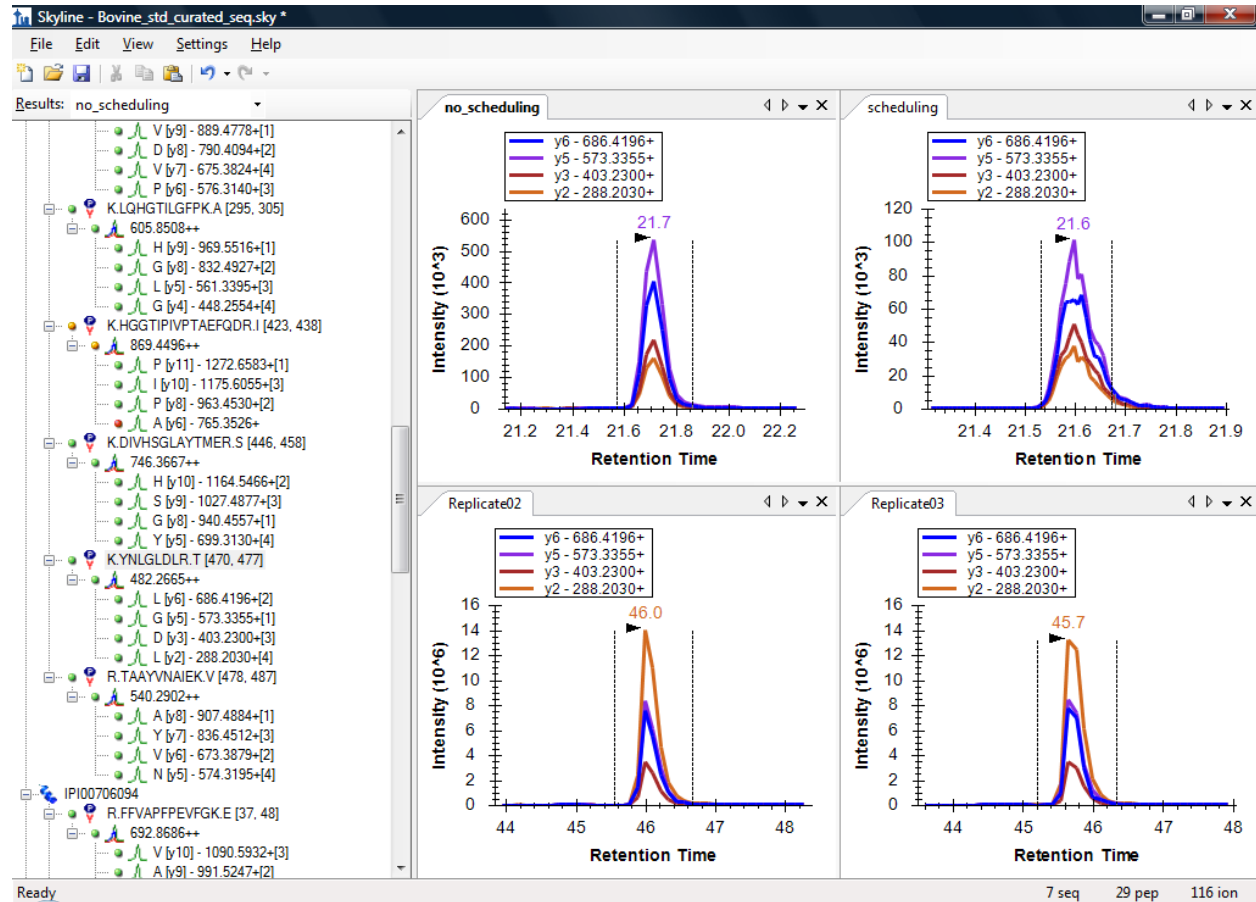


CPTAC Required Multi-Instrument Tool



▶ Applied Biosystems 4000 Q Trap

▶ Thermo-Scientific TSQ Ultra



Support Multiple Instrument Vendors

- ▶ Exporting transition lists & native methods
- ▶ Importing native instrument output files

- ▶ AB Sciex
- ▶ Agilent Technologies
- ▶ Thermo-Scientific
- ▶ Waters (with MassLynx 4.1 installed)



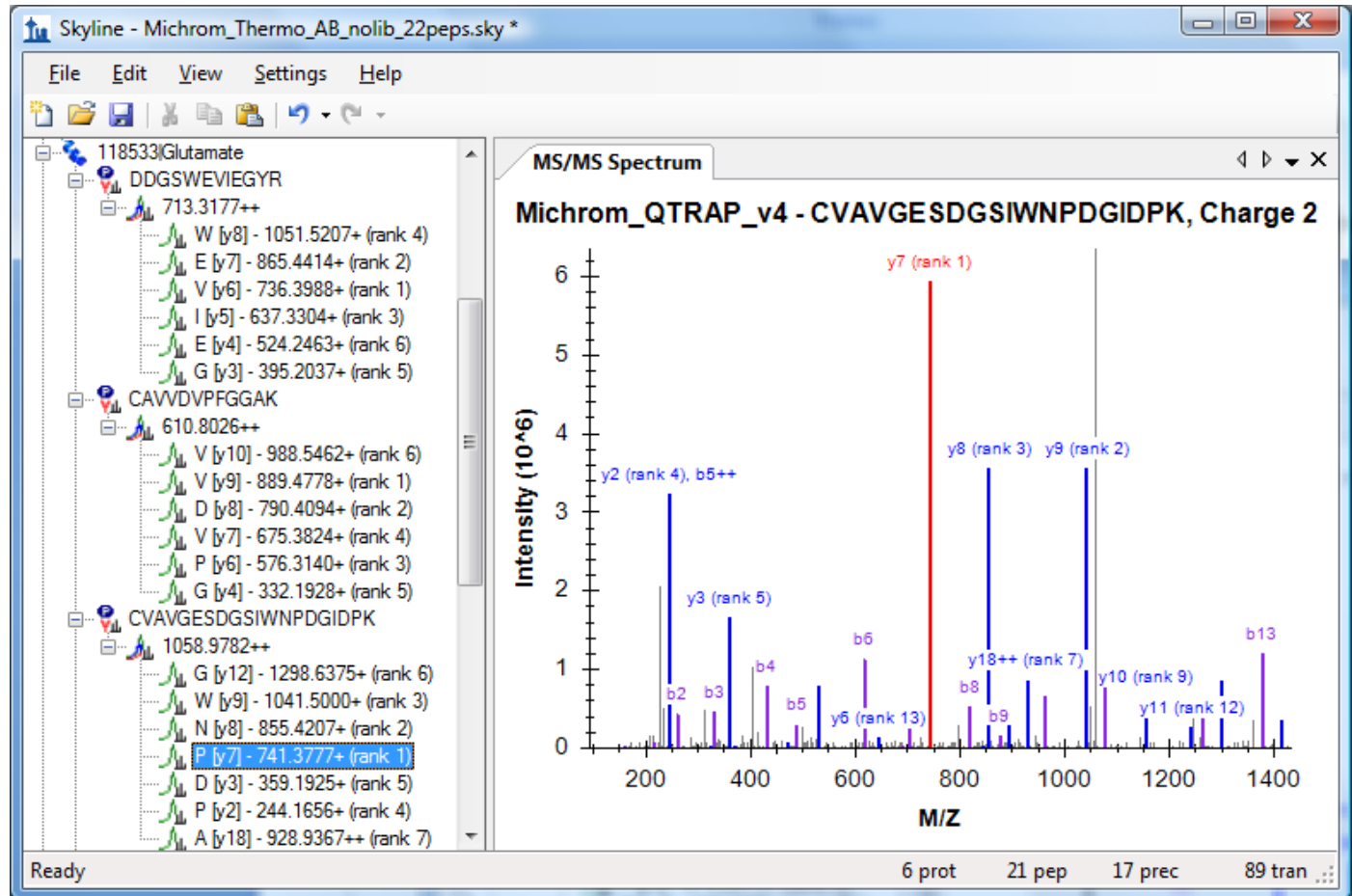
Inter-Lab Collaboration with Skyline

- ▶ NCI-CPTAC Verification Working Group Study 9S
- ▶ 12 sites
- ▶ 4 instrument manufacturers
- ▶ 7 instrument models
- ▶ Sharing
 - ▶ Methods
 - ▶ Spectral libraries
 - ▶ Data analysis
 - ▶ Reports



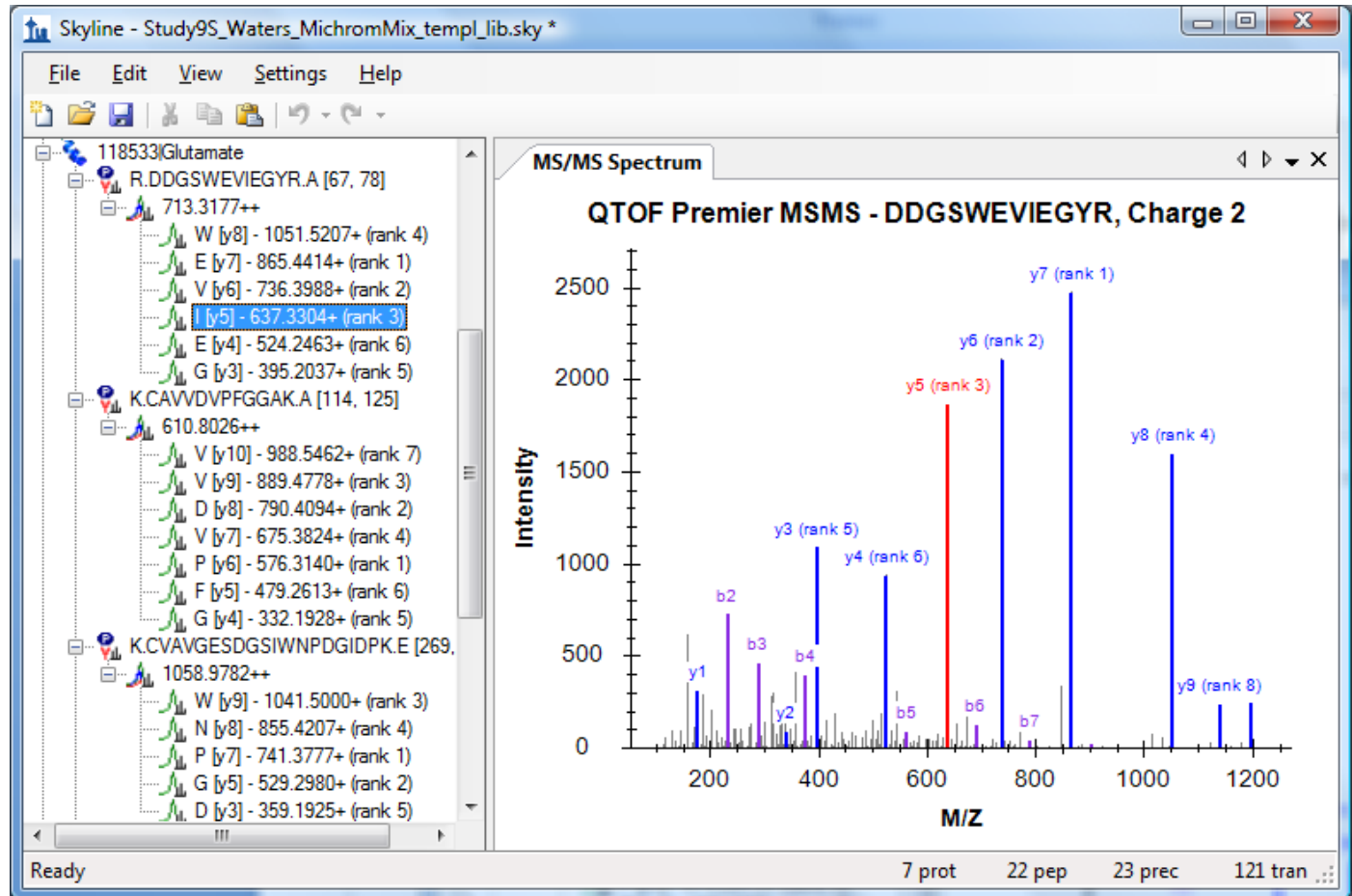
Sharing Method Creation

- ▶ With MS/MS Spectral libraries
- ▶ AB 4000 Q Trap
- ▶ Spectrum Mill



Sharing Method Creation

- ▶ With MS/MS Spectral Libraries
- ▶ Waters QTOF
- ▶ Mascot



MS/MS Spectral Library Sources

- ▶ Global Proteome Machine
- ▶ MacCoss Lab
- ▶ NIST
- ▶ Peptide Atlas

- ▶ Build your own from peptide search results
 - ▶ Mascot
 - ▶ Myrimatch / IDPicker
 - ▶ Protein Pilot (work in progress)
 - ▶ Spectrum Mill
 - ▶ TPP – pepXML / mzXML files – Peptide Atlas
 - ▶ X! Tandem



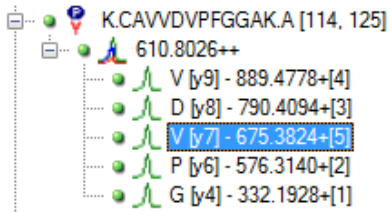
Method Creation (measure and refine)

The screenshot displays the Skyline software interface. On the left, a tree view shows a protein identified as 118533|Glutamate with a sequence KCAVDVDFGGAKA [114, 125]. Below this, a list of peptides is shown, including D [y13] - 1413.6645+, G [y12] - 1298.6375+[6], S [y11] - 1241.6161+, I [y10] - 1154.5840+, W [y9] - 1041.5000+[5], N [y8] - 855.4207+[4], P [y7] - 741.3777+[1], D [y6] - 644.3250+, G [y5] - 529.2980+[2], I [y4] - 472.2766+, and D [y3] - 359.1925+[3]. The main window shows an 'Unrefined' chromatogram with Intensity (10³) on the y-axis (0 to 180) and Retention Time on the x-axis (40.0 to 40.6). A peak is labeled with a retention time of 40.5. A 'Refine' dialog box is open on the right, with the 'Results' tab selected. It contains the following settings:

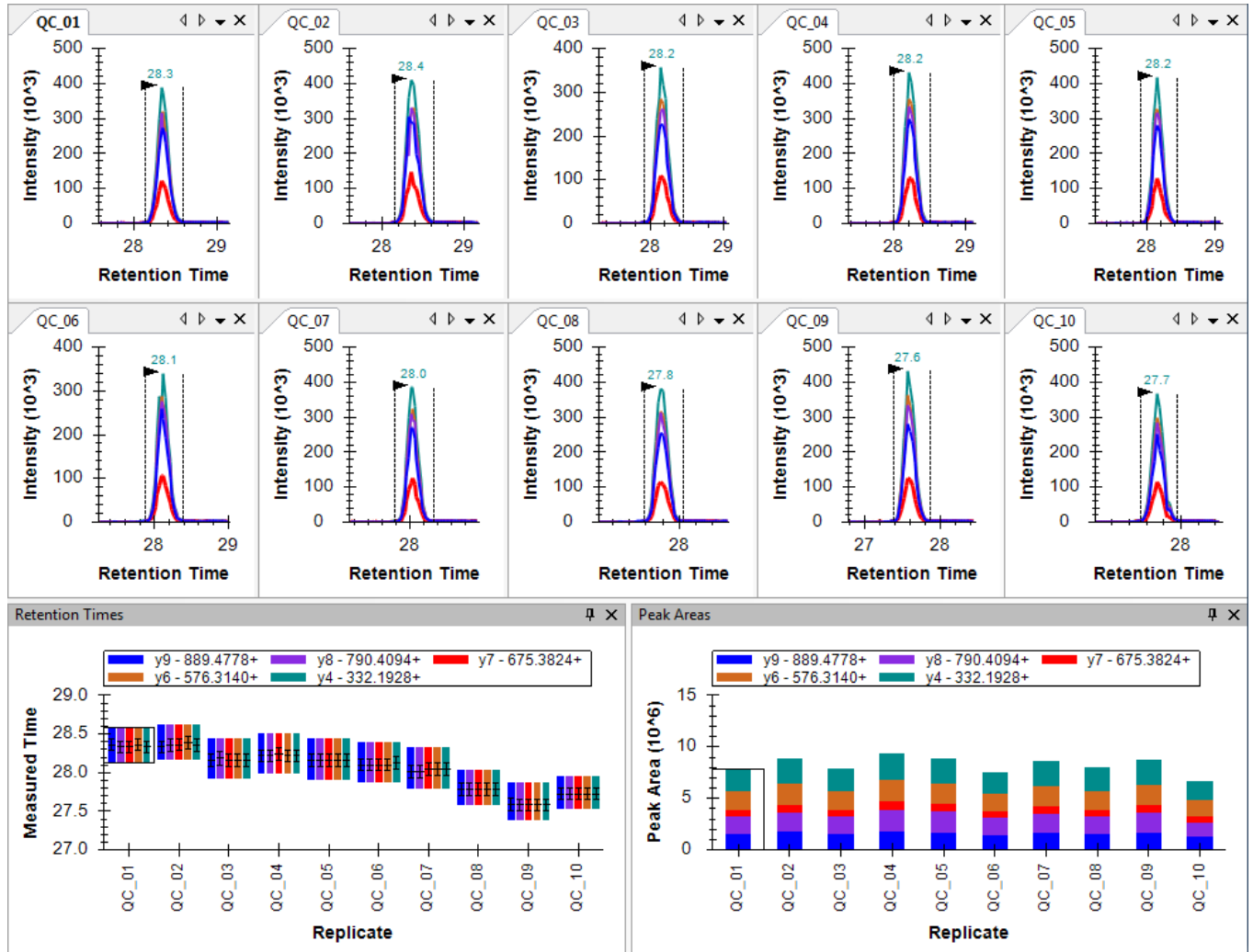
- Min peak found ratio:
- Max peak found ratio:
- Max transition peak rank:
- Prefer larger product ions
- Ignore nodes missing results
- Remove nodes missing results
- Retention time outliers:
- Target r value for linear regression:
- Spectral library correlation:
- Min dot-product:

At the bottom of the Skyline window, the status bar shows 'Ready' and '7 prot 17 pep 17 prec 118 tran ...'.

Sharing Data Analysis

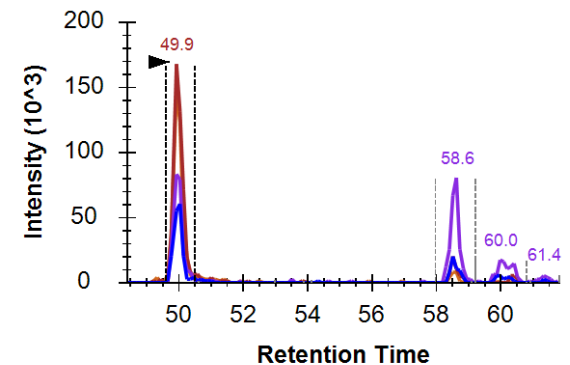
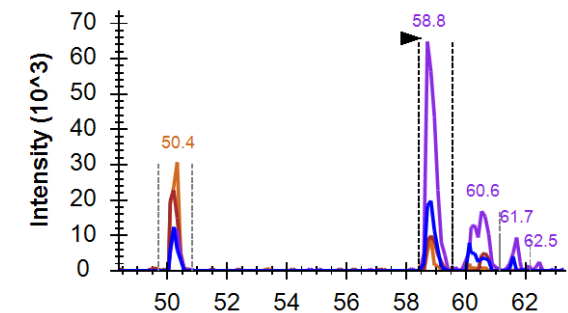
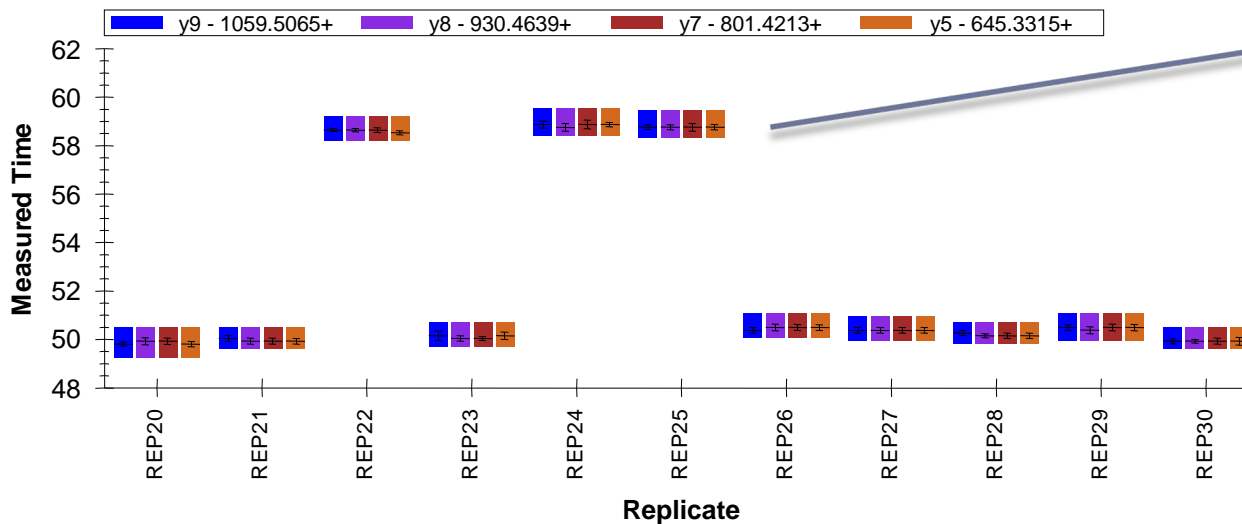
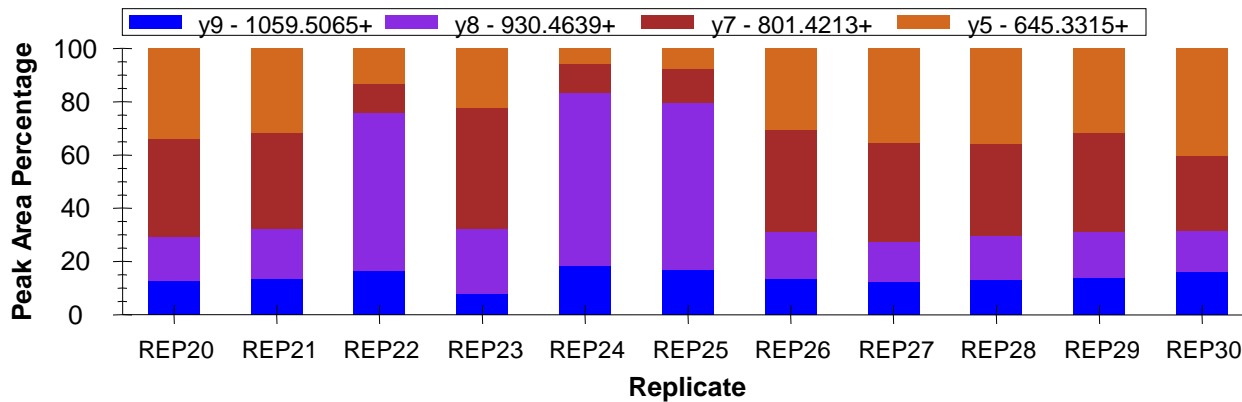


- ▶ 22 peptides
- ▶ 5 transitions each
- ▶ 10 replicates



Finding Issue Quickly (wrong peak)

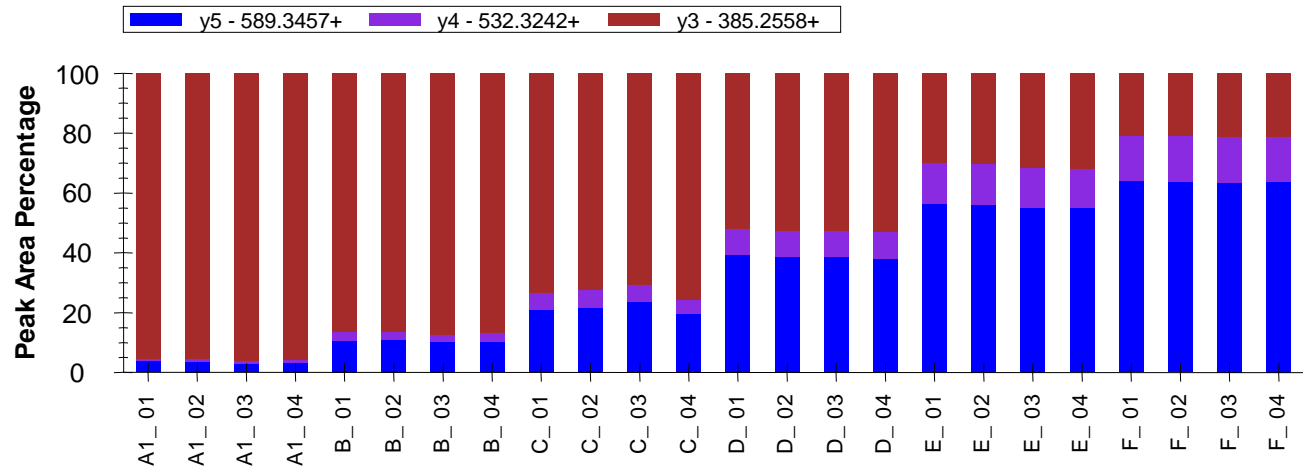
IVGYLDEEGVLDQNR



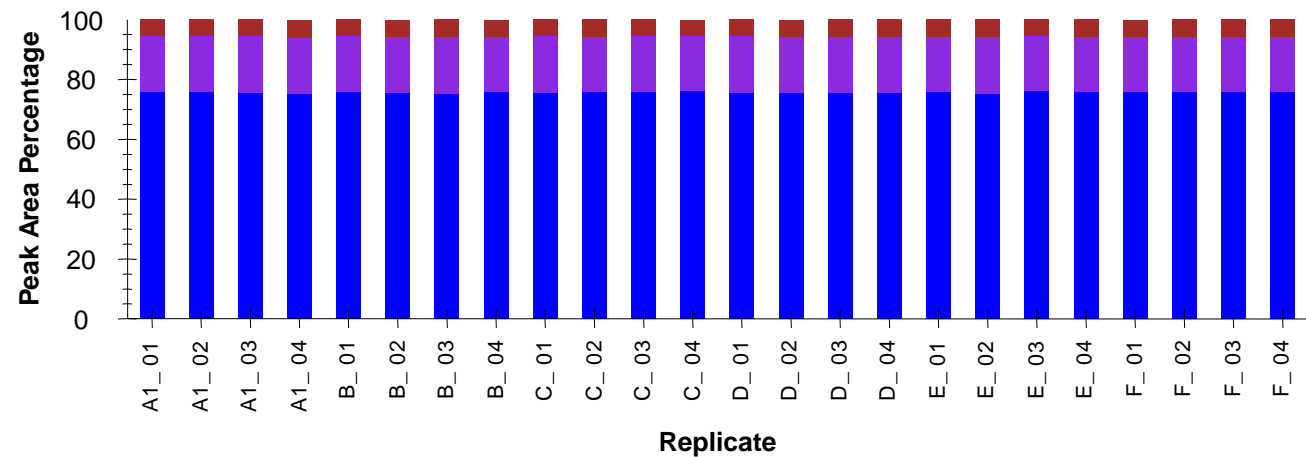
Finding Issues at a Glance (interference)

HGFLPR

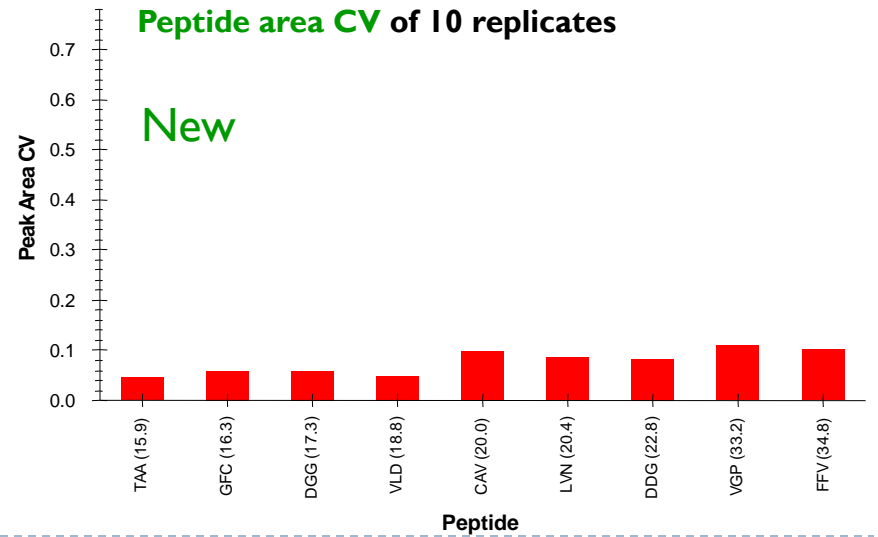
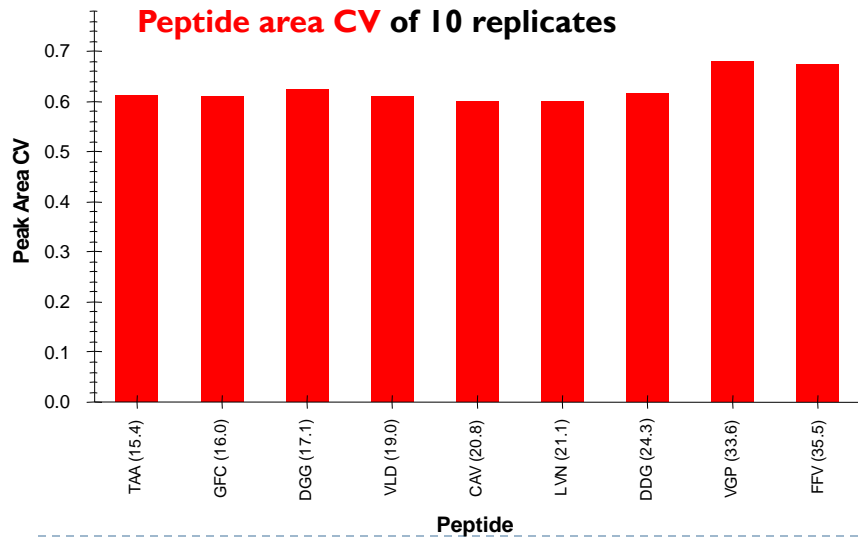
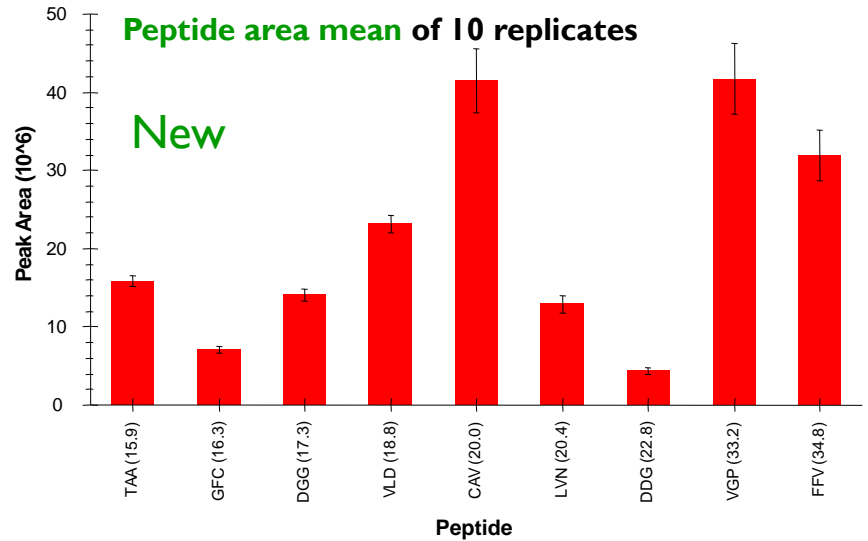
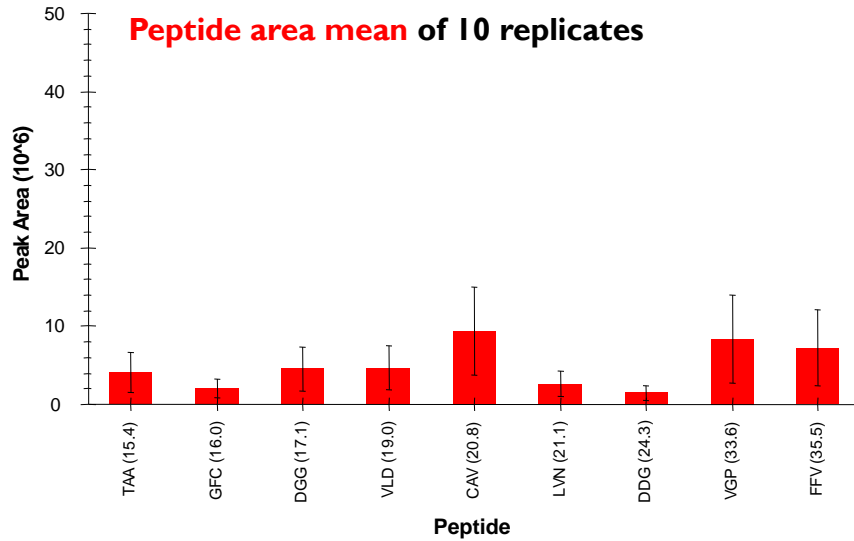
Unlabeled



Labeled
I3C R

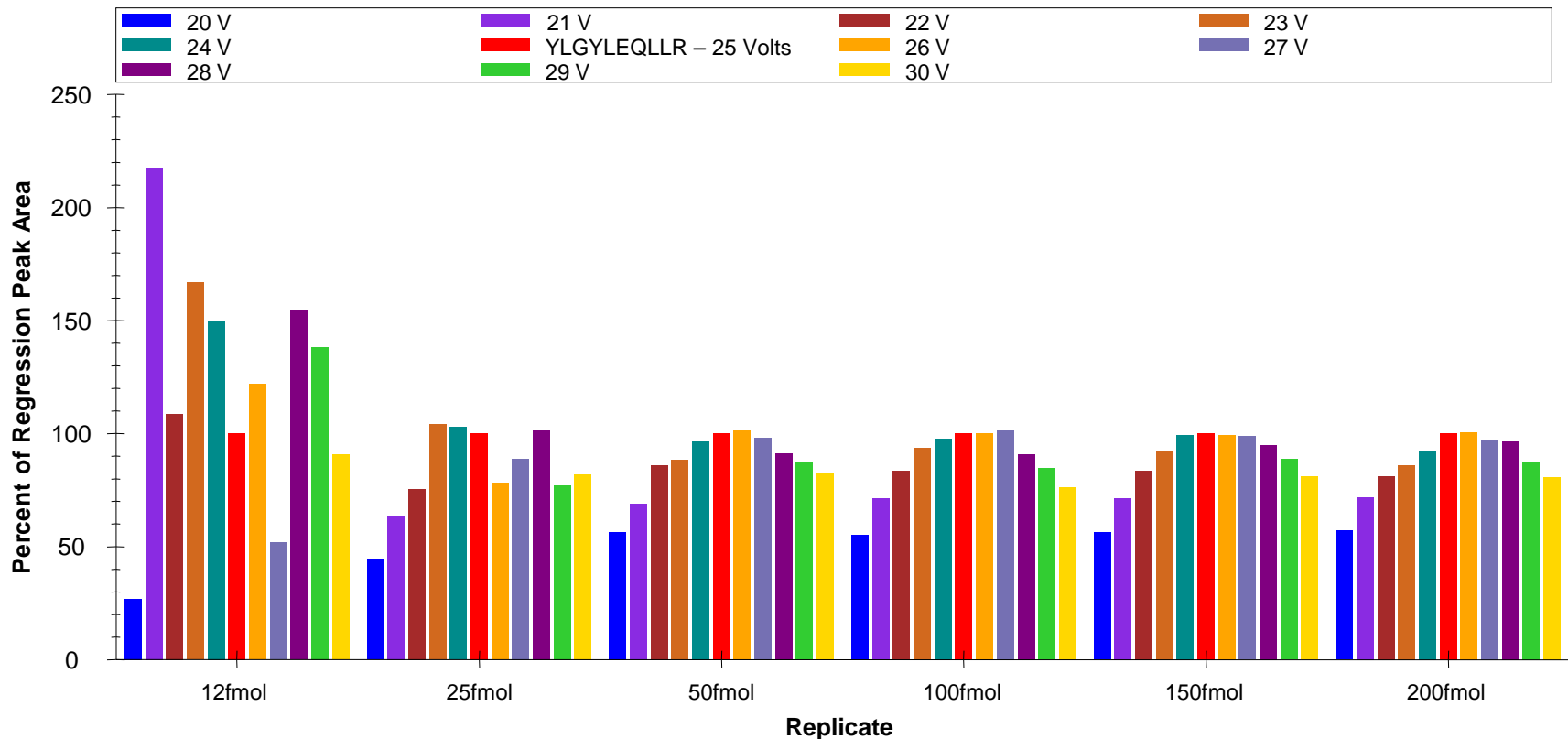


Finding Issues Early (lack of precision)



Understanding Targeted Data

- ▶ Effect of Collision Energy Optimization
- ▶ Daniela Tomazela – Wednesday AM – WPI 68



Sharing Reports

PeptideSeq	Replicate	Pre	Fragr	AverageM	PeptideRt	PrecursorI	ProductM	Retention	Fwhm	Area
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.96	533.295	966.5142	24.99	0.13	273200
VLVLDTDYK	6ProtMix_	2	y8	24.62	25.05	533.295	966.5142	25.02	0.15	291000
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.91	533.295	966.5142	24.91	0.17	220600
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.79	533.295	966.5142	24.79	0.14	331650
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.73	533.295	966.5142	24.76	0.15	346800
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.7	533.295	966.5142	24.7	0.13	265800
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.65	533.295	966.5142	24.65	0.14	301200
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.36	533.295	966.5142	24.36	0.13	290800
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.1	533.295	966.5142	24.1	0.09	170600
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.33	533.295	966.5142	24.33	0.1	147700
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.47	533.295	966.5142	24.5	0.07	900
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.5	533.295	966.5142	24.5	0.03	100
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.5	533.295	966.5142	24.45	0.03	100
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.96	533.295	853.4302	24.96	0.14	2528350
VLVLDTDYK	6ProtMix_	2	y8	24.62	25.05	533.295	853.4302	25.05	0.15	2816900
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.91	533.295	853.4302	24.91	0.16	2215900
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.79	533.295	853.4302	24.79	0.15	3202250
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.73	533.295	853.4302	24.73	0.15	3098300
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.7	533.295	853.4302	24.7	0.14	2618300
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.65	533.295	853.4302	24.65	0.13	2990900
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.36	533.295	853.4302	24.36	0.14	2794850
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.1	533.295	853.4302	24.1	0.1	1676000
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.33	533.295	853.4302	24.33	0.13	1533550

Edit Report

Report Name:

- Peptides
 - Precursors
 - Transitions
 - Results
 - PrecursorPeakFoundRatio
 - BestRetentionTime
 - MaxFwhm
 - MinStartTime
 - MaxEndTime
 - TotalArea
 - TotalBackground
 - TotalAreaRatio
 - TotalAreaNormalized
 - LibraryDotProduct
 - UserSetTotal
 - OptStep
 - OptCollisionEnergy
 - OptDeclusteringPotential
 - Note
 - Results Summary
 - Charge
 - IsotopeLabelType
 - NeutralMass
 - Mz
 - CollisionEnergy
 - DeclusteringPotential
 - ModifiedSequence

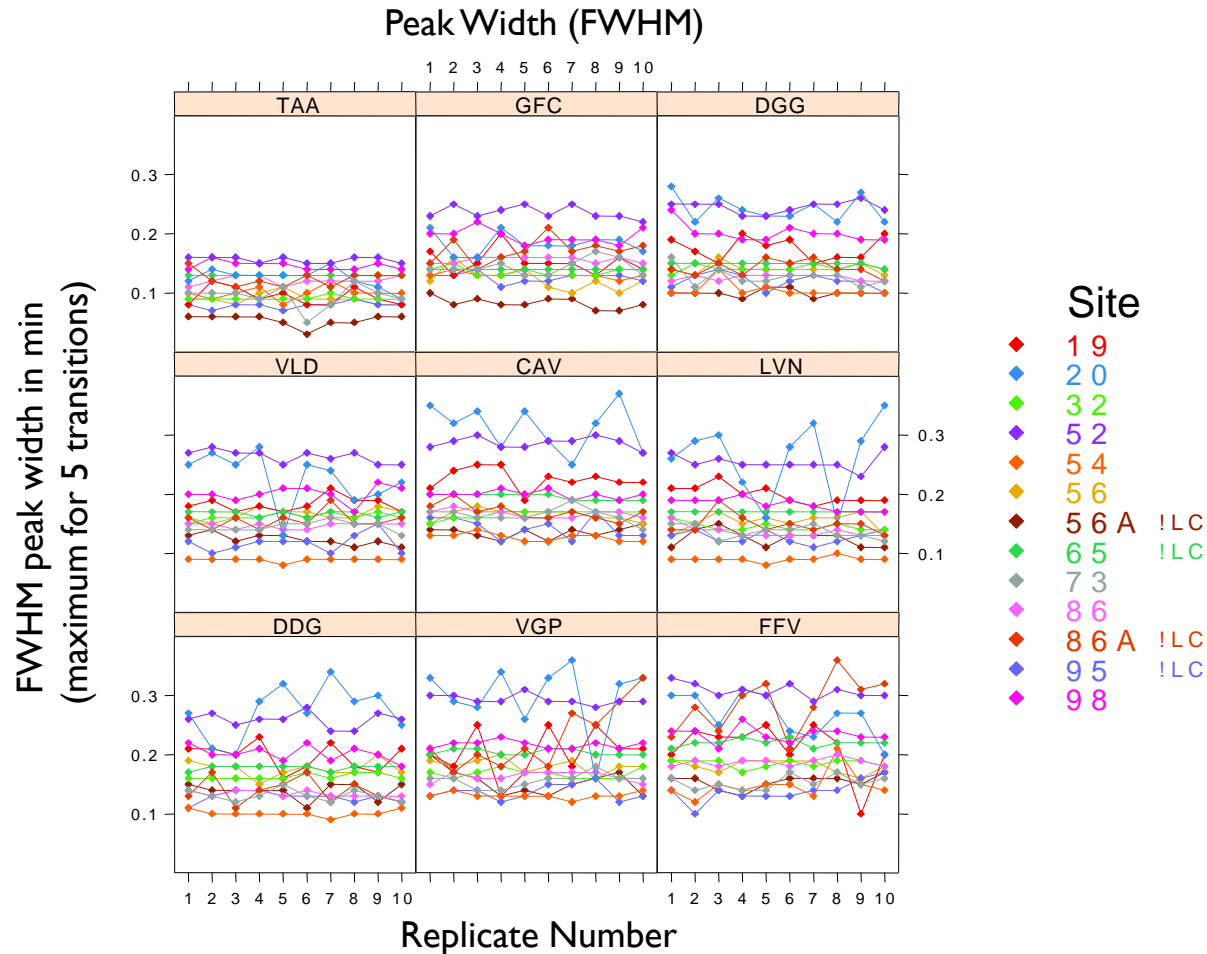
FileName
 SampleName
 ProteinName
 PeptideSequence
 ReplicateName
 PrecursorCharge
 FragmentIon
 AverageMeasuredRetentionTime
 PeptideRetentionTime
 RatioToStandard
 PeptideNote
 PrecursorMz
 ProductMz
 RetentionTime
 Fwhm
 Area
 Height
 IsotopeLabelType
 UserSetPeak
 TransitionReplicateNote
 PrecursorNote
 StartTime
 EndTime
 MinStartTime
 MaxFwhm
 MaxEndTime

Pivot Replicate Name Pivot Isotope Label



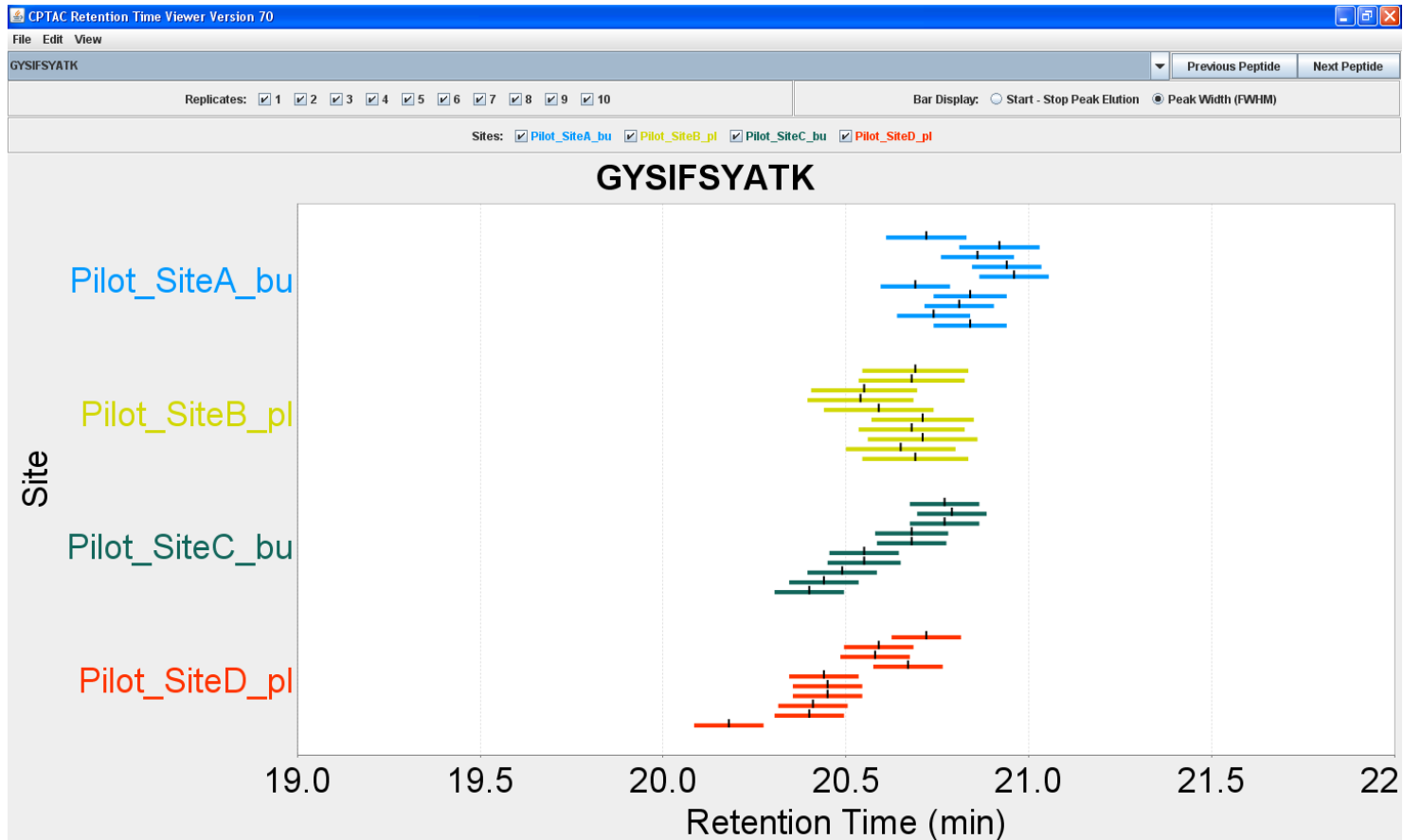
Multi-site Analysis with Statistical Tools

► Analysis of Study 9S reports with R



Deeper Analysis with Custom Tools

- ▶ Analysis of reports with Retention Time Viewer (Java program)



Summary Reports

Edit Report

Report Name:

- Peptides
 - Precursors
 - Transitions
 - Results
 - ResultsSummary
 - MinBestRetentionTime
 - MaxBestRetentionTime
 - RangeBestRetentionTime
 - MeanBestRetentionTime
 - StdevBestRetentionTime
 - CvBestRetentionTime
 - MeanMaxFwhm
 - StdevMaxFwhm
 - CvMaxFwhm**
 - MeanTotalArea
 - StdevTotalArea
 - CvTotalArea
 - MeanTotalAreaRatio
 - StdevTotalAreaRatio
 - CvTotalAreaRatio
 - MeanTotalAreaNormalized
 - StdevTotalAreaNormalized
 - CvTotalAreaNormalized
 - Charge
 - IsotopeLabelType
 - NeutralMass
 - Mz

- FileName
- ProteinName
- PeptideSequence
- PrecursorMz
- PrecursorCharge
- MinBestRetentionTime
- MaxBestRetentionTime
- RangeBestRetentionTime
- MeanBestRetentionTime
- CvBestRetentionTime
- MeanMaxFwhm
- CvMaxFwhm
- MeanTotalArea
- CvTotalArea

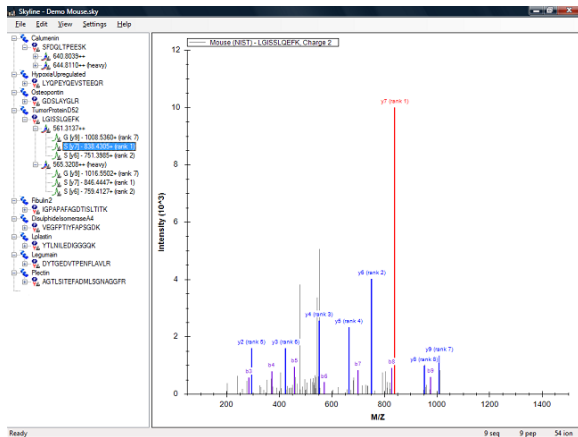
Pivot Replicate Name Pivot Isotope Label

MeanMaxF	CvMaxFwhm	MeanTotal	CvTotalArea
0.16	10.50%	6262310	40.70%
0.18	21.70%	2696540	34.30%
0.2	30%	2251420	31.20%
0.17	10.30%	10496555	16.30%
0.4	50.50%	181989	71.50%
0.19	6.80%	11882000	9.10%
0.15	7.40%	4899805	48.50%
0.18	32.60%	1621625	28%
0.18	5.90%	2926985	8.80%
MeanMaxF	CvMaxFwhm	MeanTotal	CvTotalArea
0.14	3.30%	19845013	12.30%
0.14	4.20%	7320359	13%
0.17	4.40%	14509946	11.70%
0.16	3.50%	29123865	14.20%
0.16	3.10%	2947409	13.20%
0.17	3.10%	52308520	11.90%
0.09	3.80%	16156992	23.70%
0.19	3.50%	14336958	8.70%
0.15	4.10%	17080130	17.20%

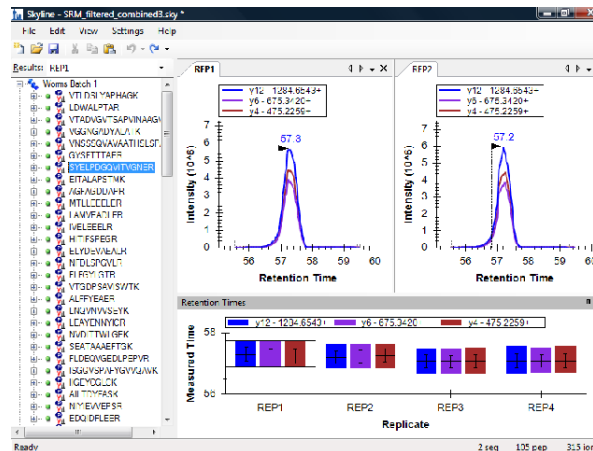
Getting Started

- ▶ Freely available & Open Source
<http://proteome.gs.washington.edu/software/skyline>
- ▶ Self-updating web installation
- ▶ 3 instructional videos (25 minutes each)

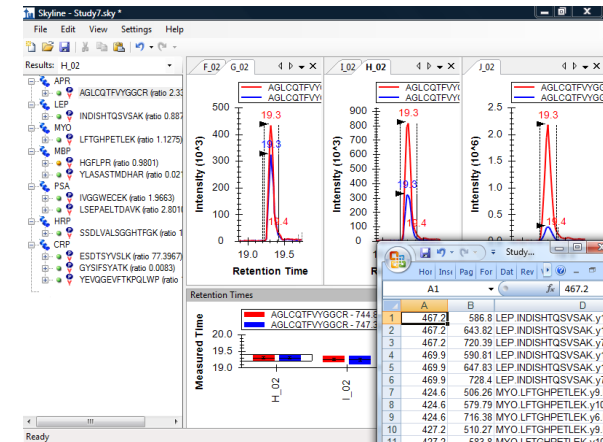
Building Methods



Results & Refinement



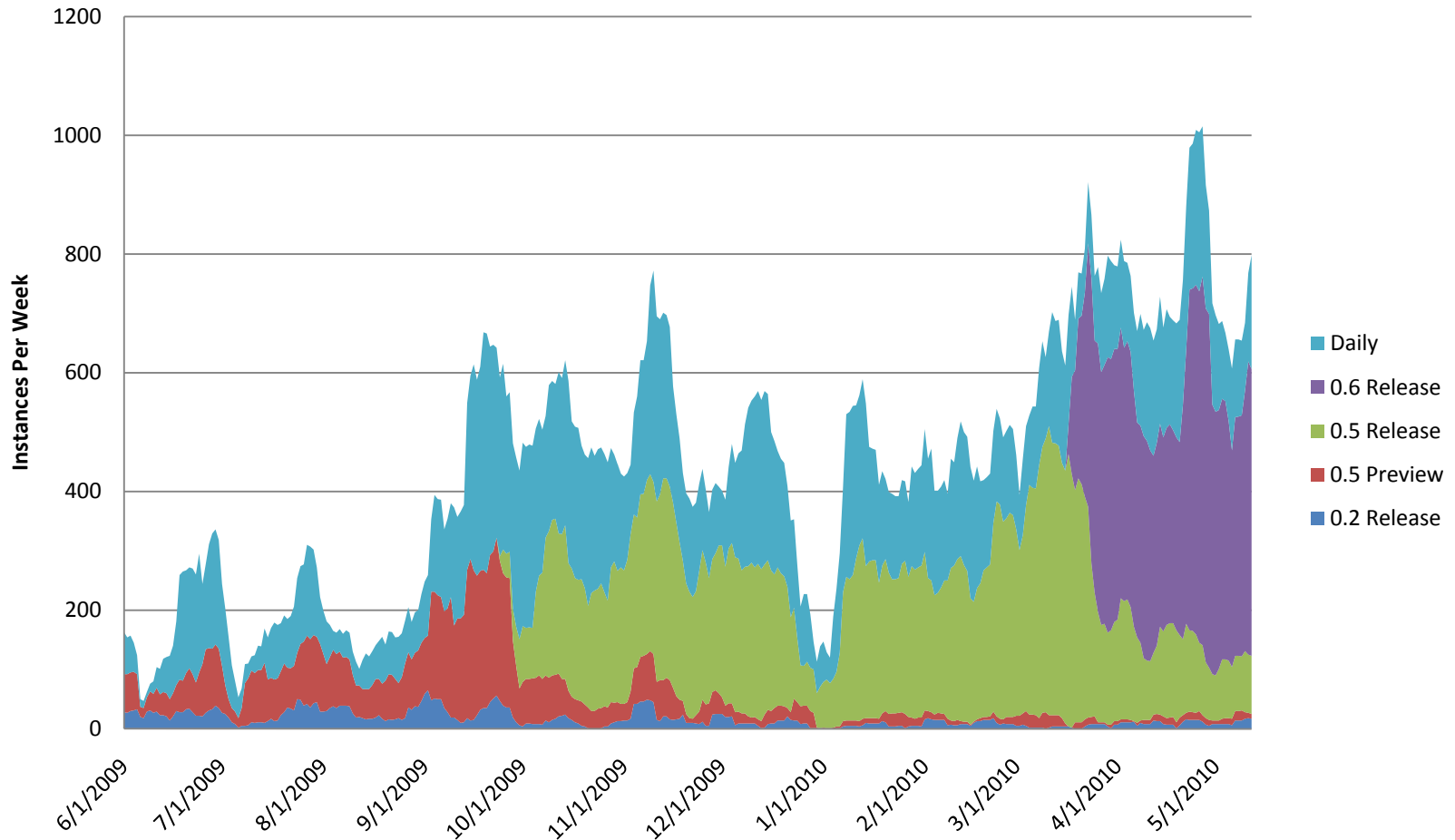
Existing Experiments



- ▶ Support board and issues list
- ▶ NEW! Tutorials with real data

Skyline Adoption (>1500 installations)

Skyline Average Usage Per Week



Acknowledgments:

- ▶ **Skyline Team**
 - ▶ Barbara Frewen
 - ▶ Gregory Finney
 - ▶ Randall Kern
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 - ▶ Xingdong Feng
 - ▶ Nell Sedransk
- ▶ **Buck Institute**
 - ▶ Michael P. Cusack
- ▶ **ProteoWizard**
 - ▶ Parag Mallick
 - ▶ Darren Kessner
- ▶ **AB Sciex**
 - ▶ Sean Seymour
 - ▶ Alpesh Patel
- ▶ **Agilent Technologies**
 - ▶ Christine Miller
- ▶ **Thermo-Scientific**
 - ▶ Tina Hemenway
 - ▶ Eric Hemenway
 - ▶ Scott Peterman
- ▶ **Waters**
 - ▶ James Langridge
 - ▶ Kieran Neeson
 - ▶ Keith Richards

All members of the NCI-CPTAC Verification Working Group

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